Prenatal Immunotoxicant Exposure and Postnatal Autoimmune Disease

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Reports in humans and rodents indicate that immune development may be altered following perinatal exposure to immunotoxic compounds, including chemotherapeutics, corticosteroids, polycyclic hydrocarbons, and polyhalogenated hydrocarbons. Effects from such exposure may be more dramatic or persistent than following exposure during adult life. For example, prenatal exposure to the insecticide chlordane or to the polycyclic aromatic hydrocarbon benzo[*a*]pyrene produces what appears to be lifelong immunosuppression in mice. Whether prenatal immunotoxicant exposure may predispose the organism to postnatal autoimmune disease remains largely unknown. In this regard, the therapeutic immunosuppressant cyclosporin A (CsA) crosses the placenta poorly. However, lethally irradiated rodents exposed to CsA postsyngeneic bone marrow transplant (i.e., during re-establishment of the immune system) develop T-cell–mediated autoimmune disease, suggesting this drug may produce a fundamental disruption in development of self-tolerance by T cells. The environmental contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) crosses the placenta and produces fetal thymic effects *in vivo* similar to effects of CsA in fetal thymic organ culture, including inhibited thymocyte maturation and reduced expression of thymic major histocompatability complex class II molecules. These observations led to the suggestion that gestational exposure to TCDD may interfere with normal development of self-tolerance. Possibly supporting this hypothesis, when mice predisposed to development of autoimmune disease were treated with TCDD during gestation, postnatal autoimmunity was exacerbated. Similar results have been reported for mice exposed to diethylstilbestrol during development. These reports suggest that prenatal exposure to certain immunotoxicants may play a role in postnatal expression of autoimmunity. *Key words:* autoimmune disease, cyclosporin A, diethylstilbestrol, prenatal, TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. — *Environ Health Perspect* 107(suppl 5):687–691 (1999).
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Prenatal immune system ontogenesis and postnatal functional integrity of the immune system require a sequential series of carefully timed and coordinated developmental events that begin early in embryonic life. In experimental animals, the developing organism has not always been found to be more sensitive to toxic effects from exposure to physical or chemical agents than the fully mature individual, but the consequences are often more severe (*1–6*). Studies in this relatively new area dealing with xenobiotic exposure during immune system organogenesis may be divided into three basic research initiatives: *a*) identification of sensitive tests and screening procedures for detecting developmental immunotoxicants; *b*) altered postnatal immunocompetence, including decreased resistance to infectious disease or neoplasia after perinatal xenobiotic exposure; and *c*) exacerbation of immune-mediated diseases, including hypersensitivity disorders and autoimmune disease resulting from perinatal exposure. Although considerable effort has been devoted to the first two of these initiatives, the possibility that developmental chemical exposure may be related to increased incidence or severity of aberrant immunity has received limited attention.

The majority of available data suggesting a link between prenatal chemical exposure and enhanced or induced postnatal autoimmunity concerns estrogenic agents such as diethylstilbestrol (DES), which alter the prenatal hormonal environment and directly target developing immune cells and halogenated aromatic hydrocarbons (HAHs) such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). The therapeutic immunosuppressant cyclosporin A (CsA) produces a T-cell–mediated autoimmunity in rodents by effects on T-cell development similar to those caused by TCDD and will also be discussed.

Consequences of Developmental Immunotoxicant Exposure

Numerous published reports indicate that developmental exposure of laboratory animals to immunotoxic chemicals may result in more severe effects on the immune system than exposure during adult life. For example, selective and persistent immune alterations have been observed in mice following gestational exposure to the organochlorine insecticide chlordane, including a significant depression of cell-mediated immunity still present 101 days after birth (*7*). Mice exposed to chlordane during fetal life also display reduced numbers of granulocyte–macrophage colony-forming units and colony-forming units in the spleen at 200 days of age (*8*) as well as long-term depression of both delayed-type hypersensitivity and mixed lymphocyte reactivity (*9*). It is noteworthy that these immune effects are either reduced or not observed in adult mice exposed to chlordane at dose levels equal to those given to the pregnant mice (*8*). Similar results have been reported in mice exposed during development to benzo[*a*]pyrene (B[*a*]P), a polycyclic aromatic hydrocarbon (PAH). For instance, offspring of pregnant mice treated with B[*a*]P have been found to display depressed antibody, graft-versus-host, and mixed lymphocyte responses at 18 months of age (*10*). These mice further exhibited an 8- to 10-fold higher tumor incidence than control mice that did not experience *in utero* B[*a*]P exposure. Low-level prenatal exposure to certain HAHs, notably dioxins, also gives rise to severe, long-lasting immunologic incompetence in rodents (*1,11,12*). Collectively, these reports demonstrate that prenatal exposure to certain immunotoxic compounds may alter fetal development of immunity in mice, causing severe and sustained postnatal immunosuppression in the absence of overt toxicity. Additional agents that produce developmental immunotoxicity in rodents are diverse and include PAHs other than B[*a*]P such as 7,12-dimethylbenz[*a*]anthracene, 3-methylcholanthrene; pesticides other than chlordane such as hexachlorocyclohexane and DDT; polycyclic halogenated hydrocarbons such as TCDD; heavy metals such as cadmium and mercury; hormonal substances such as DES, testosterone, and cortisone; mycotoxins (most notably T2 toxin); and therapeutic agents such as acyclovir and cyclophosphamide [Table 1; reviewed by Holladay and Luster (*13*)]. Several of these agents target the fetal thymus, producing fetal thymic atrophy as well as

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Table 1. Developmental immunotoxicants.[a]

Halogenated aromatic hydrocarbons
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin
Polyhalogenated biphenyls (PCBs, PBBs)
Polycyclic aromatic hydrocarbons
Benzo[*a*]pyrene
Methylcholanthrene
7,12-Dimethylbenz(*a*)anthracene
Pesticides
Hexachlorocyclohexane
Chlordane
Diazinon
DDT
Carbofuran
Fungicides
Hexachlorobenzene
Heavy metals
Methyl mercury
Lead
Cadmium
Hormonal substances
Estrogens/diethylstilbestrol
Testosterone
Cortisone
Therapeutic agents
Acyclovir
Busulfan
Cyclophosphamide
Mycotoxins
T2 toxin

Abbreviations: PCBs, polychlorinated biphenyls; PBBs, polybrominated biphenyls. [a]Modified from Holladay and Luster (*13*).

Table 2. Immunotoxicants producing fetal thymic atrophy and impaired thymocyte differentiation in mice.[a]

Immunotoxicant	Exposure regimen
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	1.5–3.0 mg/kg/day from gd 6–14
3,3',4,4'-Tetrachlorobiphenyl	6–16 mg/kg on gd 12
Diethylstilbestrol	3–8 mg/kg/day from gd 10–16
Ethylene glycol monomethyl ether	100–200 mg/kg/day from gd 10–17
Benzo[*a*]pyrene	50–150 mg/kg/day from gd 14–17
7,12-Dimethylbenz[*a*]anthracene	10–25 mg/kg/day from gd 14–17
T2 mycotoxin	1.2–1.5 mg/kg/day from gd 14–17

gd, gestational day(s). [a]Modified from Holladay and Luster (*60*).

altered differentiation of fetal T-lymphocyte precursor cells (thymocytes) (summarized in Table 2). Such chemical insult on thymocyte maturation during critical periods of self-learning may have detrimental consequences on immune function in postnatal life, including possible expression of autoimmunity (*14*). The environmental contaminant TCDD has probably received the most focused research attention in this regard.

TCDD: The Prototypic Halogenated Aromatic Hydrocarbon

The chemical agent most studied for suppressive effects on the immune system is undoubtedly TCDD. This compound has also recently become suspect for producing changes in immune cells and immune support cells, which may potentiate development of autoimmune diseases. Greenlee et al. (*15*) and Schuurman et al. (*16*) described targeting of thymic epithelium by TCDD, leading to suggestions that TCDD may have the potential to alter critical epithelium-dependent selective events in the thymus through which developing thymocytes expressing autoreactive T-cell receptors (TCRs) are deleted. DeWaal et al. (*17*) further observed altered thymic epithelial distribution of major histocompatability complex (MHC) class II molecules in TCDD-treated mice, an effect that was hypothesized as having potential to cause defective thymocyte–epithelial cell interactions. Specifically, MHC class I and class II molecules act as thymic self-antigen–presenting molecules in a process whereby thymocytes expressing TCRs with high affinity to self-antigen are eliminated (negative selection). It has also been noted that similar patterns of inhibited thymic T-cell differentiation occur spontaneously in autoimmune mice (*18*), in TCDD-treated mice (*14*), and in mice treated *in vivo* with monoclonal antibodies to MHC class I and class II molecules (*19*), suggesting the importance of these MHC molecules in thymocyte differentiation. TCDD was recently found to downregulate expression of an MHC class I gene ($Q1^b$) in a mouse hepatoma cell line (*20*). Specifically, these authors observed that MHC $Q1^b$ cDNA encoded for the α3 domain and transmembrane domain of the $Q1^b$ class I protein, implying that the MHC gene product could interact with β_2-microglobulin. These observations led to the hypothesis that the MHC $Q1^b$ molecule downregulated by TCDD may function in antigen presentation (*20*).

Together, these effects of TCDD on thymocytes, thymic epithelium, and MHC molecules associated with antigen presentation raise questions regarding the ability of TCDD to alter normal development of self-tolerance in T cells in a way that may increase expressed autoimmunity. The popliteal lymph node assay has been proposed by some as a tool to predict autoimmune reactions induced by chemicals (*21*) and recently was found to elicit a positive response in male rats injected with TCDD (*22*). Based on this observation (*22*) these authors joined others suggesting that TCDD may have the potential to induce or exacerbate autoimmunelike reactions.

Because chemical-induced altered intrathymic negative selection of potential autoreactive T cells may result in increased release of such cells to the periphery, other investigators have looked for emergence of T cells carrying TCR-variable regions associated with self-reactivity in TCDD-treated animals. The TCR-variable β (Vβ) chains are usually deleted in the thymus by reaction with self-MHC and minor lymphocyte stimulatory antigens (*23,24*) and have been associated with autoimmunity in some experimental mouse models (*25*). Therefore, deHeer et al. (*26*) examined the thymus, spleen, and mesenteric lymph nodes of adult mice dosed with TCDD for autoreactive mature $V\beta6^+$ T cells and were not able to demonstrate emergence of such cells. However, in related studies Silverstone et al. (*27*) found that both TCDD and estradiol induce extrathymic T-cell differentiation in the liver of young adult mice, and that such extrathymic cells expressed elevated levels of $V\beta^+$ TCR. Such an increase in T cells associated with autoreactivity has been suggested as a mechanism by which estrogen may promote autoimmunity (*23*). Silverstone et al. (*27*) have similarly suggested that these findings with TCDD (increased extrathymic autoreactive T cells) may relate to ability of this HAH to promote autoimmunity.

Effects of TCDD on T-Cell Development: Comparison to Cyclosporin A

The demonstration of high sensitivity of the developing immune system to TCDD, coupled with evidence that TCDD exposure in adult animals may result in increased expression of autoimmune disease, has raised questions regarding possible relationships between prenatal exposure to TCDD and increased postnatal autoimmunity. Low-level maternal TCDD exposure produces fetal thymic atrophy as well as inhibition of thymocyte differentiation (Table 3). Fetal liver T-progenitor cells seed the fetal thymus and are initially double negative with respect to CD4 and CD8 surface antigens. Subsequently, thymocytes develop sequentially through immature $CD8^{lo}$ and $CD4^+8^+$ double-positive stages in the thymic cortex to mature $CD4^+$ SP or $CD8^+$ SP thymocytes in the thymic medulla by gestational days 18–19 (*28,29*). TCDD produces a significant maturational delay in fetal thymocyte development, as evidenced by these CD4 and CD8 surface antigens. This delay has been described as similar to the maturational inhibition produced in fetal thymic organ culture by the therapeutic immunosuppressive drug CsA (*14*). Fetal thymic organ culture was used rather than *in vivo* exposure to study the effect of CsA on developing T cells because CsA crosses the placenta poorly (*30*). Fetal mouse exposure to TCDD or *ex vivo* exposure of fetal mouse thymi to CsA decreased the percentage of double-positive

Table 3. Effect of TCDD on fetal thymic weight and thymocyte CD4 and CD8 antigen expression.[a,b,c]

Treatment	CD marker expression (% positive)				Thymic weight/ body weight (%)
	$CD4^+8^-$	$CD4^+8^+$	$CD4^-8^-$	$CD4^-8^+$	
Vehicle	1.8 ± 0.2	69.1 ± 1.2	21.1 ± 0.7	8.1 ± 0.7	0.24
1.5 µg/kg	1.5 ± 0.1	52.6 ± 2.5*	30.3 ± 1.8*	15.5 ± 0.9*	0.14*
3.0 µg/kg	2.0 ± 0.2	43.2 ± 4.5*	37.3 ± 3.7*	17.5 ± 0.9*	0.10*

TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. [a]CD4 and CD8 surface antigen expression was determined in fetal mice on gestational day (gd) 18 after maternal exposure to 1.5 or 3.0 µg/kg/day TCDD from gd 6–14. [b]Values represent mean ± SEM of 5 mice per treatment group. [c]Modified from Holladay et al. (*6*). *$p < 0.05$ versus vehicle controls.

cells (the most mature phenotype present in significant numbers in the end-gestation mouse fetus) and increased the percentage of both double-negative cells and immature (i.e., TCR^-) $CD8^{lo}$ thymocytes. Any intrinsic (hormonal) or extrinsic (chemical) insult on thymocyte maturation during critical periods of thymocyte selection for self-recognition may have significant and detrimental consequences on immune function in postnatal life (*14*). Thus, this pattern of inhibition by TCDD or CsA raised questions about interference with neonatal development of tolerance by these chemicals.

In rodents, CsA also reduces MHC class II antigen expression in the thymus (*31*), an effect that was associated with development of autoimmune disease in Lewis rats exposed to CsA following lethal irradiation and syngeneic bone marrow reconstitution (*32*). Specifically, these studies demonstrated a T-cell–mediated autoimmune disease in the rats treated with CsA after bone marrow transplant, manifested as a syngeneic graft-versus-host response (SGVHR). Briefly, the development of a chronic graft-versus-host–like disease, typical of that seen in rodents or humans following allogeneic marrow transplantation, was observed in the CsA-exposed rodents that had received syngeneic bone marrow transplants (*33*). This observation of an SGVHR was the demonstration of an immune system rejecting genetic self (i.e., autoimmunity), indicating that CsA produced a fundamental disruption in development of self-tolerance. These authors demonstrated that the CsA-induced autoreactivity was transferred with the $CD8^+$ subpopulation of T cells, suggesting that CsA interfered with deletion of these cells during the establishment of a new immune system in the irradiated animals (*33*).

Model Development

The use of a bone marrow transplant model in syngeneic animals to demonstrate CsA-induced interference with normal establishment of self-tolerance may have interesting implications for future studies that consider relationships between perinatal exposure to environmental chemicals and subsequent development of autoimmune disease. Specifically, the SGVHR model mimics fetal immune development in many ways; in both cases a new immune system must be established from a limited population of hematopoietic progenitor cells. In either situation (developing fetus or adult transplant), this process requires that large numbers of immune cells be ushered through the normal selective processes governing establishment of self-recognition. It is reasonable to expect that a chemical effect on these selective processes may be more profound (thus easier to identify) in these developmental models than in an adult model in which the immune system is already established and substantially fewer immune cells are undergoing selection. Further, a syngeneic bone marrow transplant model is not limited by placental transfer of chemical. Thus the model may again facilitate identification of agents causing limited but real effects on developing immune cells. For example, placental transfer of CsA appears to be negligible (*34*), and *ex vivo* models such as fetal thymic organ culture have been used to study possible effects of this drug on immune development (*30*). Considerably less than 1% of the maternal dose of TCDD crosses the placenta in the mouse (*35*), yet this fetal exposure causes significant and diverse effects on the developing immune system (*14,36*).

The demonstration that CsA produces autoimmune disease in rodents by altering thymocyte differentiation during negative selection of autoreactive T cells, an effect that appears to be related to downregulation of thymic MHC class II molecules, brings up interesting questions regarding TCDD. TCDD produces an inhibition of fetal thymocyte differentiation very similar to that seen in fetal thymic organ cultures exposed to CsA and also interferes with expression of thymic MHC class II molecules (*17*). Thus, TCDD produces effects similar to those that have been related to production of autoimmune disease in CsA-treated animals. Whether these effects may be related to increased autoimmune disease in TCDD-exposed rodents or humans is presently not known. However, Silverstone et al. recently found that monthly exposure of young adult SNF_1 mice to TCDD resulted in the appearance of autoimmune nephritis in males in the first 6 months of life (*37*). These authors further reported that a single fetal exposure to TCDD in NZB × SWR (SNF_1) mice significantly reduced the time to postnatal onset of autoimmune nephritis in male offspring. Together, these data suggest that TCDD may have the potential to induce autoimmune disease in genetically predisposed animals. It may therefore be of interest to evaluate the effect of TCDD on development of autoimmune responses in syngeneic rodents after irradiation and bone marrow transplantation, where placental transfer does not limit exposure of the developing immune system to TCDD.

Diethylstilbestrol: A Model Estrogen

The regulatory actions of estrogenic steroids on immune function in adult animals are well documented but remain poorly understood. A role for endogenous estrogen and the development of autoimmune disease exists, as well as a correlation between increased serum estradiol levels (e.g., during pregnancy) and infections resulting from depression of cell-mediated immunity (*38,39*). Administration of pharmacologic or suprapharmacologic levels of steroidal and nonsteroidal estrogenic compounds further results in numerous alterations of immune function, particularly when administered perinatally during lymphoid organ organogenesis. Effects of such administration in rodents include myelotoxicity (*40,41*), suppression of cell-mediated immunity (*42,43*), pronounced thymic atrophy (*44,45*), depressed activity of natural killer cells (*46,47*), and stimulation of the reticuloendothelial system (*48,49*). Studies of women exposed *in utero* to DES, a synthetic nonsteroidal compound possessing estrogenic activity, suggest possible adverse affects on the postnatal human immune system. For instance, altered function of T lymphocytes and natural killer cells in women exposed to DES *in utero* has been reported (*50,51*) as well as an increased incidence of autoimmune diseases (*52*). Although it appears that estrogens mediate certain of their immune effects at the thymic level by altering thymic epithelium-dependent mechanisms (*53*), little is understood about mechanisms by which estrogenic chemicals may influence immune responses to foreign or self-antigens.

An altered prenatal hormonal environment has been associated with increased risk of developing autoimmune disease in mice (*54*). It has also been suggested that humans exposed *in utero* to DES may display a hyperreactive immune response (*55*). A retrospective study of DES-exposed (1,711 individuals) and unexposed (922 individuals) cohorts examined the possibility that prenatal DES may affect the prevalence of autoimmune disease and found a positive correlation when autoimmune diseases were grouped (*4*). Specifically, the overall frequency of any autoimmune disease among

exposed women was 28.6 per 1,000 compared to 16.3 per 1,000 among the controls (significantly different at $p = 0.02$). Autoimmune diseases evaluated included systemic lupus erythematosus, scleroderma, Graves disease, Hashimoto thyroiditis, pernicious anemia, myasthenia gravis, thrombocytopenic purpura, rheumatoid arthritis, regional enteritis, chronic ulcerative colitis, multiple sclerosis, chronic lymphocytic thyroiditis, Reiter syndrome, and optic neuritis. When these autoimmune diseases were considered individually, however, only Hashimoto thyroiditis occurred significantly more often in the exposed women ($p = 0.04$). A similar evaluation of 1,173 humans exposed to DES during development (1,079 daughters and 94 sons) found increased asthma, arthritis, and diabetes mellitus compared to prevalence rates for these diseases in the general population (*56*). However, in a more recent study evaluating rates of allergy, infection, and autoimmune disease in DES-exposed sons and daughters (253 men and 296 women) matched with similar unexposed individuals (241 men and 246 women), no differences in disease occurrence were detected (*57*). These authors concluded that a larger sample was needed to evaluate DES-associated risk of autoimmunity, since autoimmune diseases are relatively rare in the human population.

Thus preliminary studies of humans exposed before birth to DES suggest the possibility of postnatal immune alterations, including increased autoimmune disease. Continued surveillance of DES sons and daughters will be required for more definitive statements but will become more difficult as this cohort ages and members are lost. Laboratory rodent studies will therefore be important to determine if prenatal exposure to chemicals such as DES may alter development of immune cells in such a way as to predispose an individual to expression of postnatal autoimmunity and to answer questions regarding specific immune cell targets and mechanisms of action.

Hybrid $B6C3F_1$ (C57Bl/6N × C3H) mice exposed to 8 µg/kg/day DES from days 10–16 of gestation displayed significant thymic hypocellularity in late gestation as well as limited but significant inhibition of thymocyte maturation (Table 4) (*58*). Thymic involution in these studies was related to a reduction by DES of fetal liver prothymocytes responsible for colonizing the fetal thymus. These authors also reported that fetal liver prothymocytes expressed estrogen receptors at about 290 fmol/100 µg DNA, a level approximately half that found in the uterus and sufficient to suggest an estrogen-responsive cell. These and other reports indicate the developing mouse immune system is sensitive to estrogen exposure, and that such exposure may contribute to postnatal immunosuppression (*13*). However, as with most of the well-established rodent developmental immunotoxicants, very limited information is available addressing possible relationships between gestational DES exposure and altered expression of postnatal autoimmune disease.

Table 4. Effect of DES on fetal thymocyte cellularity and CD4 and CD8 antigen expression.[a,b,c]

Treatment	CD marker expression (% positive)				Cellularity ($\times 10^{-6}$)
	$CD4^+8^-$	$CD4^+8^+$	$CD4^-8^-$	$CD4^-8^+$	
Vehicle	4.1 ± 0.2	71.4 ± 0.7	22.0 ± 0.7	2.7 ± 0.2	40.4 ± 2.6
3 mg/kg	3.2 ± 0.4	64.0 ± 2.5*	28.8 ± 2.5*	4.0 ± 0.3*	18.9 ± 1.9*
8 mg/kg	4.0 ± 0.4	63.6 ± 1.6*	29.2 ± 1.5*	3.3 ± 0.3	8.7 ± 1.9*

DES, diethylstilbestrol. [a]CD4 and CD8 surface antigen expression was determined in fetal mice on gestational day (gd) 18 after maternal exposure to 3 or 8 µg/kg/day DES from gd 10–16. [b]Values represent mean ± SEM of 5 mice per treatment group. [c]Modified from Holladay et al. (*58*). *$p < 0.05$ versus vehicle controls.

A single fetal exposure to DES in SNF_1 mice induced autoimmune lupuslike nephritis in male offspring between 5 and 10 months of age (*37*). Female SNF_1 mice develop this autoimmune syndrome spontaneously in their first year of life; however, male mice do not display significant autoimmunity before 1 year of age. These data suggest that pharmacologic exposure to DES may contribute to early expression of autoimmunity in genetically predisposed mice, and that autoimmune rodent models such as the SNF_1 model may prove valuable for identification of biologic markers for human risk assessment. Because autoimmune diseases are multifactorial (genetic, environmental, hormonal, infectious) (*59*), clearly, continued research will be required to determine possible relationships between prenatal estrogen exposure and postnatal development of autoimmune disease.

Conclusion

It has been repeatedly demonstrated that the developing immune system is highly sensitive to known adult immunotoxicants that cross the placenta. However, the developmental immunotoxicity data presently available in the literature are almost exclusively limited to postnatal immunosuppression (rather than hypersensitivity or autoimmunity). Indeed, very few immunotoxicants (e.g., DES, TCDD) that have been associated with induced or exacerbated autoimmunity in adult animals have been evaluated for similar effects following gestational exposure. Preliminary data suggest that both DES and TCDD may alter development of the immune system in mice genetically predisposed to autoimmune disease, causing earlier expression of autoimmunity. DES is a nonsteroidal estrogen, and as such is often used to study the effects on immune function of both endogenous estrogens and environmental compounds possessing estrogenic activity. TCDD is the most biologically potent member of a family of polyhalogenated aromatic hydrocarbons that contains other important environmental contaminants such as polychlorinated biphenyls and halogenated dibenzofurans. Given the large number of estrogenic environmental contaminants (including many agents presently grouped under the umbrella of endocrine-disrupting compounds) and the extent of environmental contamination with TCDD and TCDD congeners, the importance of determining if these agents (acting alone or in mixtures) may alter normal development of the immune system in a way that may contribute to increased autoimmune disease in humans is becoming evident.

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